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Interaction of Vimentin With Actin and Phospholipids

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Vimentin intermediate filaments are a major cytoskeletal constituent of cells of mesenchymal origin. They have been colocalized with a variety of intracellular structures such as actin filaments and the plasma membrane. Labeled actin filaments, observed *in vitro* by fluorescence microscopy, break in the presence of polymerizing vimentin; the time course is consistent with stopped-flow measurements of vimentin polymerization. This breakage phenomenon appears to be specific for vimentin. Inhibition of vimentin network formation was observed with phosphatidyl inositol phosphate (PI(4)P) and phosphatidyl inositol bisphosphate (PI(4,5)P_2), but not phosphatidyl choline (PC), phosphatidyl serine (PS), or phosphatidyl inositol (PI). Taken together, these results indicate a specific interaction of vimentin with F-actin and polyphosphoinositide lipids.

Introduction

Vimentin-type intermediate filaments are a major cytoskeletal constituent of cells of mesenchymal origin. Theories as to their function vary from maintenance of cellular integrity (Lazarides, 1980) to gene regulation (Traub and Shoeman, 1994). There is *in vivo* evidence for vimentin colocalization with other cytoskeletal elements, such as actin (Brown and Binder, 1992; Cary *et al.*, 1994; and Tint *et al.*, 1991) and microtubules (Gurland and Gunderson, 1995; Gyoeva and Gelfand, 1991), as well as with cellular organelles, such as the plasma membrane and the nucleus. Here we present *in vitro* studies indicating that vimentin interacts directly with both F-actin and polyphosphoinositide lipids.

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Materials and Methods

Purification of actin and vimentin

Actin was purified by the method of Spudich and Watt (1971) with slight modifications. Actin was stored in G-buffer (2 m*M* Tris, 0.2 m*M* $CaCl_2$, 0.5 m*M* ATP, 0.5 m*M* DTT, pH 8.0) at −80°C; it was polymerized at a concentration of 5 μM by the addition of a ten-times concentrated solution of F-buffer (1×:20 m*M* Tris, 150 m*M* KCl, 2 m*M* $MgCl_2$, 0.2 m*M* $CaCl_2$, 0.5 m*M* ATP, 0.5 m*M* DTT, pH 7.4) and was stabilized by the addition of equimolar TRITC-phalloidin (Sigma Chemicals, St. Louis, MO). Vimentin was purified from Ehrlich ascites tumor cells by the method of Nelson *et al* (1982). Vimentin was extensively dialyzed against non-polymerizing buffer (10 m*M* Tris, 6 m*M* DTT, pH 7.6) to remove residual urea and was polymerized by the addition of KCl to 150 m*M*. All reagents were purchased from Sigma Chemicals (St. Louis, MO).

F-actin-vimentin interactions

F-actin, under either polymerizing or non-polymerizing conditions, and stabilized by TRITC-phalloidin (10 n*M*), was visualized by fluorescence microscopy in the presence of 20 μM unpolymerized vimentin. A large number of fields were recorded to videotape over time, and the average length of F-actin—based on at least 200 filament traces—was calculated.

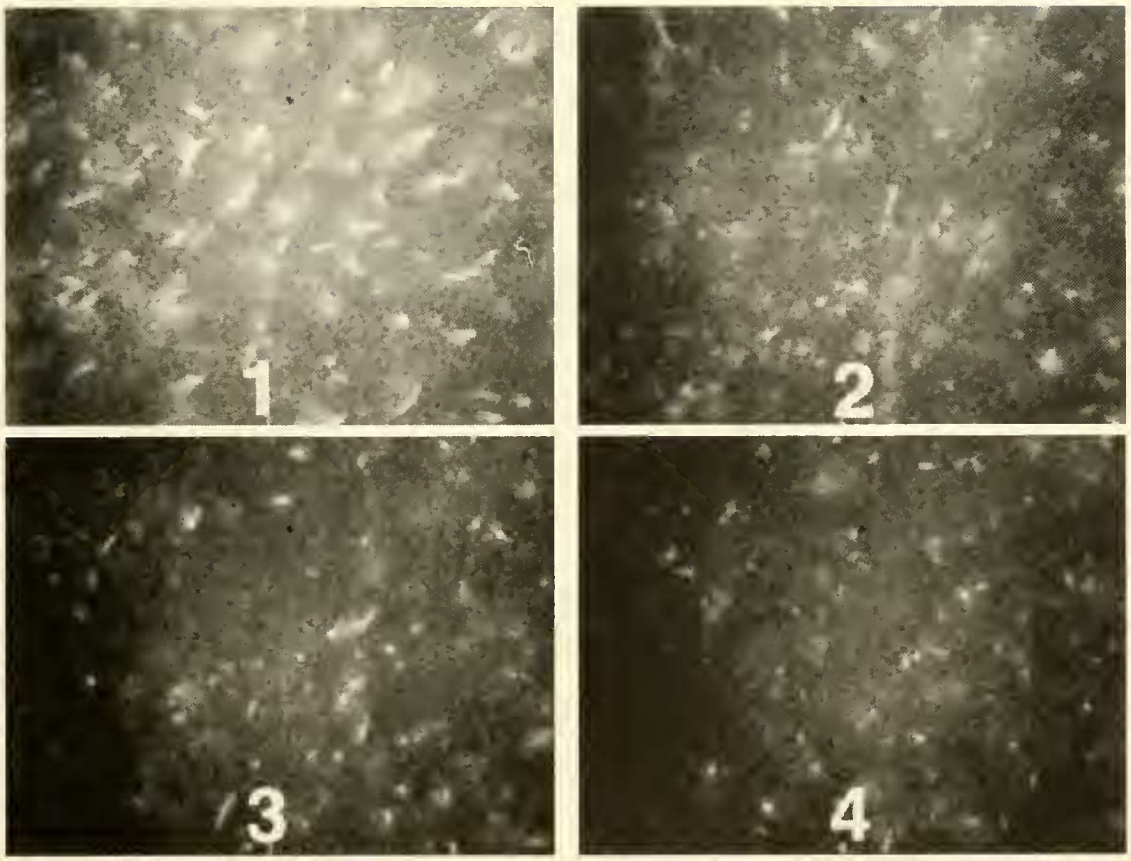

Figure 1. With increasing time, the average length of F-actin decreases in the presence of polymerizing vimentin. Labeled, phalloidin-stabilized actin filaments (10 n*M*) are visible by fluorescence microscopy in a sample containing 18 μM (1 mg/ml) unlabeled vimentin before and after addition of KCl to initiate vimentin polymerization. The dimension of each image is 50 μm in width, and the image in panel 4 was taken 25 min after polymerization.

Rheology of vimentin networks

Rheology of vimentin networks was carried out as described previously (Janmey *et al.*, 1991). Oscillatory measurements of elastic modulus were made with a Rheometrics RFS II fluids spectrometer (Rheometrics, Piscataway, NJ) at a frequency of 1 rad/s and a strain amplitude of 1%.

Results

F-actin-vimentin interactions

Labelled actin filaments were examined *in vitro* by fluorescence microscopy and were seen to break in the presence of polymerizing vimentin. Figure 1 shows a panel of four video frames; the fluorescent filaments are F-actin in a matrix of polymerizing unlabeled vimentin filaments. Such breakage was not seen under non-polymerizing conditions, indicating that polymerization is required. The decrease in average F-actin length is shown

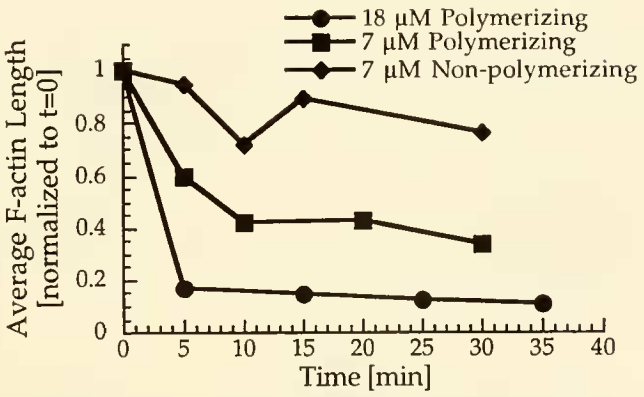

Figure 2. Average length of F-actin decreases in the presence of polymerizing vimentin filaments. Higher concentrations of vimentin result in faster rate of length decrease. Unpolymerized vimentin does not cause a decrease in F-actin length.

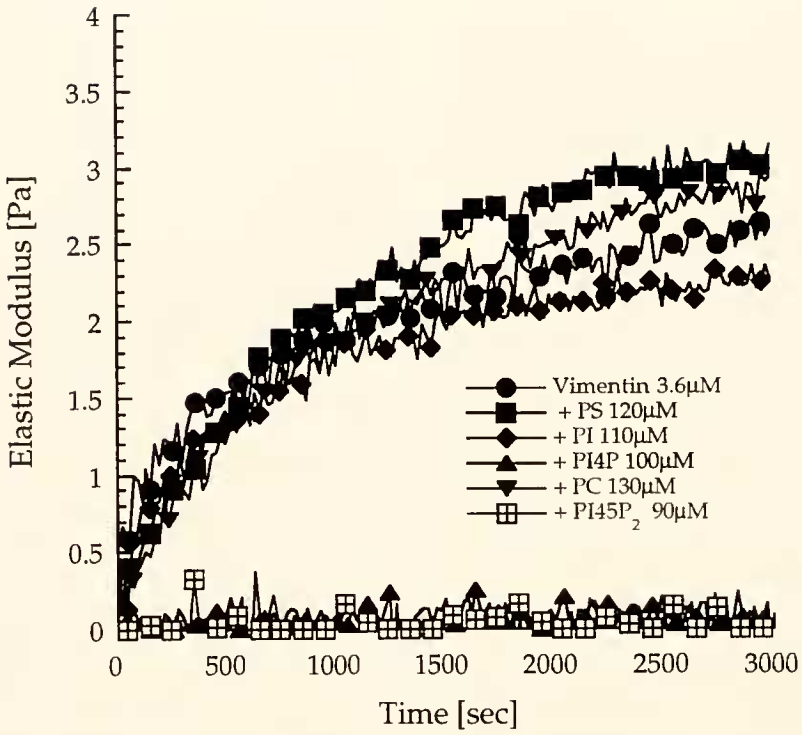

Figure 3. Rheology of vimentin networks disrupted by polyphosphoinositide lipids. Other phospholipids, such as phosphatidyl serine, phosphatidyl choline, and phosphatidyl inositol, had no significant effect on vimentin polymerization.

in Figure 2 as a function of vimentin polymerization and concentration. The time course is consistent with stopped flow measurements of vimentin polymerization (data not shown). This breakage phenomenon appears to be specific for vimentin, since no breakage is seen with microtubules in actin, or with actin in microtubules or fibrin (data not shown).

Vimentin-phospholipid interactions

The interaction of vimentin and phospholipids was measured by rheological methods and showed that polyphosphoinositide lipids inhibit the formation of an elastic network. Inhibition was observed with PI(4)P and PI(4,5)P_2, and to a lesser extent with PI (Fig. 3). Inhibition was not observed with PC or PS. These results are consistent with early studies by Perides *et al.* (1986) showing that phospholipid vesicles, especially those containing PIP and PIP_2, inhibit vimentin polymerization and depolymerize preformed vimentin filaments.

Conclusions

Fluorescent actin filaments decrease in length in the presence of polymerizing vimentin. Filament breakage was not observed in other biopolymer systems, indicating that the interaction is specific.

PIP and PIP_2 inhibit the polymerization of vimentin as measured by rheological methods. This evidence points to a specific interaction between vimentin and polyphosphoinositide lipids.

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